A STUDY OF THE BACTERIAL FLORA OF THE FECES AND INTESTINAL CONTENTS OF THE FOWL

THESIS FOR THE DEGREE OF M. S.

Mark Wirth Emmel

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A STUDY OF THE BACTERIAL FLORA OF THE FECES AND INTESTINAL CONTENTS OF THE FOWL.

Thesis

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THESIS

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I. INTRODUCTION

For many years it has been known that the intestinal tract of man and animals is the habitat of millions of micro-organisms. Comparatively little work has been directed to the intestinal flora of the fowl especially in-so-far as a systematic study is concerned. Many workers have reported various organisms as occurring in the feces of the fowl but none have attempted a study of the percentage occurrence of the organisms present. It appears that such a study would be of extreme importance as enteritis, apparently due to many factors, frequently occurs. The fact that this condition often becomes chronic would lead one to believe that the bacterial flora of the feces and intestinal contents of the fowl may be important in such cases and may throw a new light on certain factors which have here-to-fore been overlooked.

II. Historical

Kern (1) found twenty one species of bacteria in the intestines of twenty four birds investigated by him. A common characteristic of the bacteria isolated was their ability to liquefy gelatin. A relatively large number reproduced by spores. Only a few produced color and these were principally micrococci. Bacillus coli was the predominating organism.

Although Schottelius (2,3,4) did not identify organisms of the intestinal flora, he found that the presence of micro-organisms in the intestinal canal was necessary for the normal development of the chick. He concluded from his classical experiments, in which chicks were hatched, fed and grown under strictly sterile conditions, that the micro-flora of the digestive tract assists in the resolution of food consumed, that substances which stimulate peristalsis by their irritating action on the intestinal wall are produced, and, that the normal flora destroys and prevents proliferation of pathogenic organisms which gain access to the intestinal canal.

King (5) found the flora of the intestinal mucosa to be to a certain extent constant. This flora appears to depend upon the environment and varied within cer-

tain limits according to external conditions. Bacillus coli was the predominating organism and it was almost always found in the ceca and colon, frequently found in the lower portion of the small intestine and rarely found in the duodenum.

Gage (6) found that the intestinal flora of 48 healthy fowls varied to some extent with the conditions of environment and different stages of life. Bacillus coli was the predominating organism. Few obligate anaerobes were present.

Rahner (7) found Esch. coli, gram-positive cocci, molds, Bacillus megatherium and lactic acid bacteria in the intestines of hens. He thinks that Esch. coli is a constant inhabitant and tends to crowd out other organisms whose nature depends to a great extent upon the food consumed. Esch. coli increased in number as the cloaca was approached.

Menes and Rochlin (8) found the flora of hens to contain but few species of bacteria and to be identical in all parts of the intestines. The uniformity of the micro-flora is explained by the presence of energetic acid builders. This they think also explains the absence of putrefactive bacteria and their associated decomposition processes.

III. THE BACTERIAL FLORA OF THE FECES OF HEALTHY MATURE FOWLS.

PROCEDURE. Samples of fresh feces were collected in sterile test tubes from the cloaca of apparently healthy birds. A portion about the size of a pea from each sample was transfered to a tube containing 5 c.c. of sterile nutrient broth. The mixture was thoroughly emulsified. Dilutions of 1 to 100, 1 to 500, 1 to 1000, 1 to 10,000, and 1 to 100,000 were plated on nutrient agar medium. All samples were diluted and plated in duplicate. The plates were then incubated for 48 hours at 37°C. In most instances all of the colonies were picked from the plates. In certain instances when desirable plates were not obtained all of the colonies were picked from a representative portion of the plate. Transfers were made to slants of nutrient agar and incubated for 48 hours at 37°C. The slants were then replated and incubated for 48 hours at 37°C after which the organisms were transfered to nutrient agar slants. These slants were kept as stock cultures, transfers being made from them for work on identification.

All of the colonies growing aerobically on the original plates were marked. These plates were then placed in anaerobic jars and incubated for 7 days at 37°C in order to isolate any obligate anaerobes which might be present.

The organisms, after being obtained in pure culture, were first stained by Burke's modification of the Gram stain (9) and streaked on nutrient agar slants in order to study their morphological and cultural characteristics. Bergey's Manual (10) was followed in a large measure in identifying the organisms although frequently it was necessary to resort to other sources (Weldin (11) and Ford (12)) for a more detailed description of specific organisms.

Acid and gas fermentation studies were first made on dextrose, lactose and sucrose peptone water media. These data together with those already obtained were sufficient in many instances to place the organisms in their proper genus. Growth on mannite, maltose, arabinose, raffinose, dulcite, dextrin, salicin, rhamnose, xylose,and sorbitol peptone water media as well as tryptophane broth, litmus milk and nitrate solution were very useful in identifying many of the organisms. However, it was necessary to resort to other media for the identification of certain organisms.

The acetyl-methyl-carbinol test of Voges and Proskauer (13) and the methyl red reaction as described by Clark and Lubs (14) were used in differentiating between the genera Escherichia and Aerobacter. There proved to be a very marked correlation between these tests. This corresponds to the findings of Levine (15), Clark (16) and others.

RESULTS. The occurrenceof Escherichia coli in the feces of these hens varied from 5.4 to 78.3 per cent, the average occurrence of this organism being 33.66 per cent. Escherichia communior varied in the feces of the same hens from 3 to 78.8 per cent, the average occurrence of this organism being 31.02 per cent. The occurrence of these two organisms collectively rarely fell below 40 per cent in individual birds. Escherichia neapolitana and Escherichia acidi lactici occurred in the feces of two hens while Escherichia alcalescens occurred in the feces of but one bird.

Staphylococcus citreus occurred in the feces of eight birds. While this organism was not present in great abundance it occurred more frequently than any organisms with the exception of Esch. coli and Esch. communior, its occurrence varying in individual birds from 3.1 to 20.4 per cent. Staphylococcus albus occurred in the feces of but three birds.

Organisms of the genus Bacillus occurred very frequently but usually in small numbers. Often several species were found in the feces of the same bird. B. mycoides and B. subtilis were most prevalent, each occurring in the feces of five birds. B. cereus occurred in the feces of four birds, while B. cytaceus, B. tritus, B. circulans, B. megatherium, B. petasites, B. ramosus, B. cohaerens and B. vulgatus each occurred in the feces of one bird.

Table No.2.

Percentage occurrence of organisms in the feces of healthy mature birds.

Organism	Bird 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Esch. coli	31.5	78.3	30.3	5.4	46.4	22.7	13.2	50.9	32.7	37.2	32.6	25.6	18.9	12.4	20.0	18.7	44.5	60.6	50.7	40.6
Esch. communior	20.0	10.6	13.0	69.2	38.2	15.1	22.7	28.3	26.3	21.3	34.0	37.7	78.8	26.5	17.5	32.4	53.4	15.6	38.9	30.9
Micro. aurantiacus	3.2																			
Staph. albus	6.8	2.2					5.4													
Rhod. agilis	2.5																		9.5	
Unidentified #	36.0					5.4	38.3													
B. mycoides		8.9						15.6	7.0				2.3						2.1	
Esch. neapolitana			16.1		3.4															
Esch. acidi lactici			40.6		3.4															
Sal. icteroides				25.4		30.2														
B. cytaceus					8.6															
Sarcina subflavus						16.5														
Esch. alcalescens						10.1														
Staph. citreus							5.2		15.1		6.1	9.4		20.4	4.7	3.1				12.4
B. tritus							10.1													
Bact. bifidus							5.1					4.1								
Aer. aerogenes								5.2												6.1
B. subtilis								12.6			12.1	16.3				12.1				10.0
Clos. sporogenes								6.3												
B. circulans										1.9										
B. megatherium										20.0										
Achromo. liquifaciens										16.4				8.1	10.1					
Act. microflavus										3.2	2.2									
B. mesentericus											13.0									
B. cereus												6.9			3.7			8.3	8.3	
B. petasites														28.2						
Act. ruber														4.4						
Lacto. acidophilus															25.5					
B. ramosus															8.4					
Micro. subflavus															10.1					
B. cohaerens																13.5				
Act. bobili																20.2				
B. vulgatus																	2.1			
Micro. percitreus																		6.0		
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Apparently the same organism in each bird.

Aerobacter aerogenes was found in the feces of but two birds and constituted, respectively, 5.1 and 6.1 per cent of the total intestinal flora.

Two obligate anaerobes were found in the feces of the twenty birds, Bacteroides bifidus occurred twice while Clostridium sporogenes occurred but once.

Achromobacter liquifaciens occurred in the feces of three birds. Salmonella icteroides and Actinomyces microflavus occurred in the feces of two birds while the remaining organisms occurred but once in the feces of various birds.

An organism not identified occurred in the feces of three birds. It represented, respectively, 5.4, 38.3 and 36 per cent of the total flora. The behavior of this organism was so nearly alike in each instance that it seems highly probable that all three cultures were identical. The organism constantly stained negative by the Gram stain but showed a very high per cent of involution forms when grown on various media. Its motility and physiological activities on different media varied so greatly from time to time that identification was impossible.

IV. THE BACTERIAL FLORA OF THE FECES OF TWO WEEKS OLD HEALTHY CHICKS.

PROCEDURE. The method of procedure was the same as that followed in determining the bacterial flora of the feces of healthy mature birds.

RESULTS. The presence of Esch.coli and Esch. communior in the flora of the feces of the baby chicks studied appeared to be rather variable. Esch. coli was absent in the feces of two of the ten chicks while Esch. communior was absent in the feces of one chick. Esch. coli was most abundant in the feces of chick No. 4 where it constituted 74.1 per cent of all organisms present. In the feces of chick No. 1 80.7 per cent of the organisms proved to be Esch. communior. The average per cent of Esch. coli found in the feces of the ten chicks was 26.3, while the average per cent of Esch. communior was 33.01 . Esch. acidi lactici occurred in the feces of four chicks and constituted from 10.7 to 30.2 per cent of the total flora. Esch. leporis occurred in the feces of one chick.

Staphylococcus citreus and Staphylococcus albus occurred in the feces of two chicks while Staphylococcus aureus occurred in the feces of one chick.

Organisms of the genus Bacillus occurred in the feces of many of the chicks but usually represented a small percentage of the total flora. B. mycoides was found in the feces of six chicks, B. mesentericus and

Table No.1.

Percentage occurrence of organisms in the intestinal contents of chicks.

Oragnism	Chick									
	1	2	3	4	5	6	7	8	9	10
Esch. coli		24.5	8.2	74.1	41.6	28.1		41.2	7.1	41.2
Esch. communior	80.7	36.8	44.8	2.0	26.2	36.4	63.5	12.4		27.3
Phyto. vitians	10.0									
Staph. aureus	4.5									
Staph. albus	0.5								12.3	
B. mycoides	2.5	15.3	8.2	10.0		11.0			30.4	
Serratia havanaensis	0.4									
Micro. subflavescens	1.4									
B. lactis		12.2								
B. petasites		7.6								
Sarcina subflava		2.1								
Serratia marcescens		0.5								
B. flavus		1.0								
Esch. acidi lactici			30.2	12.7				11.2		10.7
Aer. aerogenes			4.5				20.4			
Esch. leporis			4.1							
Flavo. denitrificans				1.2						
B. vulgatus					11.4					
Rhod. rosaceus					8.5					
Staph. citreus					12.3			14.6		
Micro. aurantiacus						10.0				
Achromo. rugosum						8.2				
Pseudo. fluorescens						6.3				
B. subtitis							9.5			5.4
B. cohaerens							6.6			
B. mesentericus								8.1	15.2	
Sarcina lutea								5.8		
Serratia lactici								6.7		
B. terminalis									19.8	
B. circulans									15.2	
Esch. neapolitana										7.9
Act. citreus										2.1
Act. albus										2.4
Bact. liquifaciens										3.0
	100	100	100	100	100	100	100	100	100	100

B. subtilis were found in the feces of two chicks while B. lactis, B. petasites, B. flavus, B. vulgatus, B. terminalis and B. circulans each occurred in the feces of but one chick.

Aerobacter aerogenes was found in the feces of two chicks to the extent of 4.5 and 20.4 per cent respectively.

The remaining organisms occurred but once in the feces of the various chicks.

V. THE BACTERIAL FLORA OF THE INTESTINAL CONTENTS OF TEN CHICKS AFFECTED WITH PULLORUM DISEASE.

PROCEDURE. A sample of the intestinal contents was placed in 5 c.c. of sterile physiological salt solution. In instances where no contents were readily available the mucosa was scraped with a sterile scalpel, the scrapings being placed in 5 c.c. of sterile physiological salt solution. In either case the samples were thoroughly emulsified and a procedure similar to that employed in determining the bacterial flora of the feces of healthy mature birds was followed. It is regretable that no record was kept as to the exact location from which the samples were taken as Mallmann (17) has shown that there may be considerable variation in the presence of Salmonella pullorum at different levels of the intestinal tract. Sal. pullorum was isolated from the various organs of the ten chicks with the exception of chicks No. 2 and No. 8. No attempt was made to isolate any obligate anaerobes which might be present.

RESULTS. The occurrence of Esch. coli and Esch. communior in the intestinal contents of these ten chicks did not correspond to their occurrence in the normal chicks studied. Esch. coli was absent in the intestinal contents of two chicks and varied in the remainder from 4.1 to 30 per cent, being present in an average of 10.9 per cent. An average of 2 per cent of the flora of the

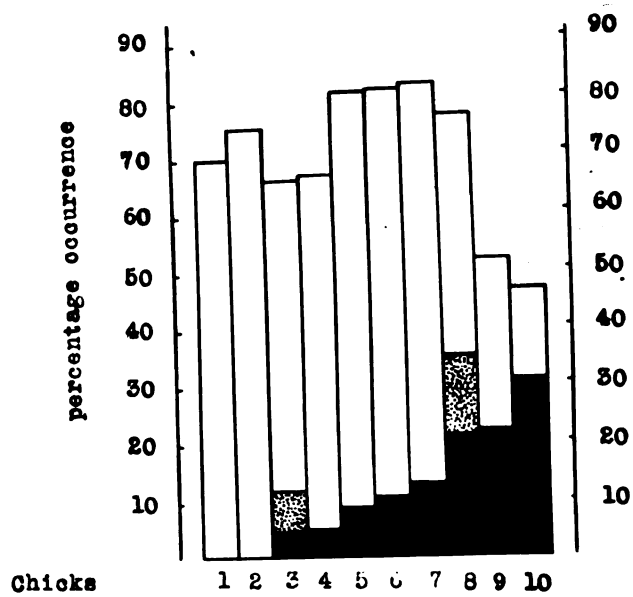

Chart No. 1. Percentage occurrence of Sal. pullorum, represented in white, Esch. coli, represented in black, and Esch. communior, shaded, in the feces of ten two week old chicks affected with pullorum disease.

of the intestinal contents of ten chicks consisted of Esch. coli, this organism being absent in eight chicks and constituting 13.9 to 6.9 per cent of the flora of the intestinal contents in the remaining two chicks.

There was also a marked reduction in the prevalence of spore spore bearing organisms in the intestinal contents of these ten chicks as compared to the normal chicks studied. Spore bearers constituted an average of 3.9 per cent of the total organisms present. B. mycoides occurred in the intestinal contents of two chicks while B. subtilis, B. ramosus and B. fusiformis occurred but once in the intestinal contents of the ten chicks.

There seemed to be very little change in the prevalence of Staphylococcus in the intestinal contents of the above chicks. Staph. citreus and Staph. aureus both occurred in the intestinal contents of two chicks while Staph. albus occurred in the intestinal contents of but one chick.

An interesting feature of the bacterial flora of the intestinal contents of these chicks was the presence of organisms of the genera Eberthella, Proteus, Salmonella and Streptococcus.

Sal. pullorum was the predominating organism in the intestinal contents of all of the chicks with the exception of one. The occurrence of this organism ranged from 16.1 to 74.3 per cent of the total organisms present in the flora.

Table No.3.

Percentage occurrence of organisms in the intestinal contents of chicks affected with pullorum disease.

Organism	Chick									
	1	2	3	4	5	6	7	8	9	10
Esch. coli	20.1	30.0	4.1	4.5	10.0	12.5		20.3	8.0	
Esch. communior	13.9		6.9							
Sal. pullorum	42.5	16.1	54.6	61.8	71.5	69.1	74.8	30.5	73.1	69.9
Strep. lactis	11.2									
Staph. aureus	12.3							10.0		15.2
Esch. acidi lactici		4.8		14.5						
Achromo. candicans		10.0								
B. ramosus		19.8								
E. ambigua		19.3								
Proteus vulgaris			21.2							
Staph. albus			8.0							
Aero. aerogenes			5.2							
B. fusiformis				7.1						
Staph citreus				12.1		13.4		3.5	3.5	
Esch. neapolitana					4.5					
Strep. bovis					8.6					
Strep. liquefaciens					5.4			19.2		
Achromo. liquidum						5.0				
Strep. pyogenes							20.1			
Salmonella							5.1			
B. subtitis								7.1		
B. mycoides								12.9		3.4
Eberthella									15.4	
Proteus valeriae										11.5
	100	100	100	100	100	100	100	100	100	100

VI. THE BACTERIAL FLORA OF THE INTESTINAL CONTENTS OF TWENTY ADULT BIRDS AFFECTED WITH ENTERITIS.

PROCEDURE. The method of procedure was similar to that followed in determining the bacterial flora of the intestinal contents of the ten chicks affected with pullorum disease. It was necessary to secure more samples from scrapings from the mucosa as diarrhea was a common symptom in these birds and the intestinal tract was in most instances devoid of abundant contents. No record was kept as to the exact location from which the samples were taken and no attempt was made to isolate obligate anaerobic organisms which might be present.

RESULTS. There was a marked variance in the prevalence of Esch. coli and Esch. communior in the intestinal contents of these twenty hens as compared to the flora of the feces of apparently healthy birds. Esch. coli was absent in the intestinal contents of eight birds while Esch. communior was absent in the intestinal contents of six birds. Esch. coli represented an average of 12.2 per cent of the flora of the intestinal contents of the twenty birds. Esch. communior comprised an average of 10 per cent of the total flora in the same birds.

B. cereus, B. mycoides, B. subtilis, B. megatherium, B. cohaerens, B. fusiformis and B. ramosus occurred in the intestinal contents of the various birds and repre-

sented an average of 3.3 per cent of the total flora.

Staph. aureus and Staph. albus represented an average occurrence in the intestinal contents of these birds of 5.5 per cent, occurring in the flora of four and six birds respectively.

The presence of Strep. lactis, Strep. citrovorus, Strep. pyogenes and Strep. bovis constituted an average of 4.6 per cent of the organisms present in the intestinal contents, occurring in 5, 1, 2, and 1 birds respectively.

As a rule the majority of the organisms making up the flora of the intestinal contents of these birds belonged to the genera Salmonella and Eberthella. An average of 20.9 per cent belonged to the former genus while an average of 33.5 per cent belonged to the genus Eberthella.

Table No.4.

Percentage occurrence of organisms in the intestinal contents of twenty hens affected with enteritis

Organism	Bird																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Esch. coli	22.2		18.9		21.6	12.9	22.8			45.7	9.1	4.0	4.6	34.6		5.6		42.4		
Esch. communior	22.8		9.4		10.2		11.1			32.8	10.4		20.9	10.4			10.5	44.2		16.2
Strep. lactis	22.3										2.5	20.3							5.3	
B. mycoides	18.6			8.6														6.2		
B. subtilis	14.1	8.0																3.8		
Proteus vulgaris		18.1		21.4																
Eberthella		73.9		43.8																
Staph. albus			19.4				9.0				13.2				15.2		6.8			
Salmonella			39.2					52.9		12.6		67.4		24.9				3.4		56.9
Alcaligines recti			13.1																	
Achro. liquifaciens				16.2	21.1			5.0		5.3										
Achro. liquida				10.0																
B. cereus					18.1				6.5											
Flavo. brevis					19.1															
Strep. pyogenes					9.9											15.2				
Strep. bovis						13.4														
Eberthella						15.4														
Salmonella						48.2					44.0									
Achro. album						10.1														
Salmonella							20.1													
Sal. pullorum							24.6													
Staph. aureus							10.6													
B. fusiformis								10.5												
Eberthella								31.6												
Eberthella									61.4											
Eberthella									32.1											
B. ramosus										3.6										
Pseudo. fluorescens											10.8									
Staph. aureus											10.0									
Staph. citreus																				26.9
Strep. citrovorus												8.3								
Eberthella													44.6							
Eberthella													29.9		12.7	29.2				
Eberthella														30.1		46.9	20.4		68.6	
Eberthella															72.1		62.3			
B. cereus																3.1				
B. megatherium																			1.5	
Salmonella																		22.6		
B. cohaerens																			2.0	
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

CELLULOSE DECOMPOSITION

PROCEDURE. As cellulose decomposing organisms have been found in the feces of practically all domesticated animals an experiment was set up to study the cellulose decomposing organisms occurring in the feces of the normal adult bird.

Samples of fresh feces were collected from the cloaca of fifty hens in sterile test tubes. A portion of feces the size of a large pea was transfered from each sample to test tubes containing Omeliansky's medium (18). After transfering the feces to the medium one c.c. of sterile oil was placed on the surface of the medium although it was later found that the addition of this oil was unnecessary providing the tube was tightly sealed. The tubes were then incubated at 37°C overnight after which they were allowed to stand at room temperature.

RESULTS. Readings were taken at seven day intervals as indicated in table No.5. The first evidence of cellulose decomposition occurred at the end of the sixth week. Additional tubes showed evidences of cellulose decomposition until the fifteenth week after which 17 tubes remained in which decomposition failed to take place. At this time the filter paper in 33 tubes was completely wilted and many of them completely decomposed.

Microscopic examination of smears from the medium revealed a variety of organisms. However, in most of the

Table No.5.

CELLULOSE DECOMPOSITION

Time	Condition of filter paper		
	No change	Slightly wilted	Completely wilted
	No. of tubes	No. of tubes	No. of tubes
7 days	50		
14 "	50		
21 "	50		
28 "	50		
35 "	50		
42 "	48	2	
49 "	40	8	2
56 "	31	15	4
63 "	27	8	15
70 "	24	5	21
77 "	20	7	23
84 "	17	8	25
91 "	17	5	28
98 "	17	2	31
105 "	17	0	33
112 "	17	0	33

tubes gram negative rods and gram positive cocci prevailed. The cocci always seemed to be in closer contact with the decomposing filter paper while the rods seemed in greater abundance in suspension in the medium. In many tubes involution forms, while not in abundance, were considerably in evidence. Spores were numerous in many tubes, while Actinomyces were abundant in a few. All attempts to isolate the organisms responsible for the decomposition of the cellulose in pure culture failed. While many of the organisms of the fecal flora are not capable of growth in this medium, they are no doubt able to survive.

It seems that more than one species of organism or symbiotic relationship of organisms was responsible for the decomposition of the cellulose as the filter paper in all of the tubes was apparently not attacked in the same manner. In some tubes, translucent spots surrounded by a yellowish brown ring appeared, in others, the filter paper became fringed at the edges, while in others, the surface of the paper became fuzzy. In most of the tubes the sediment tended to become black or slate gray probably due to the fact that hydrogen sulphide was formed in most of the tubes.

It is interesting to note that repeated transfer of small pieces of filter paper or at least 1 c.c. of the liquid portion of the medium after the filter paper began to wilt greatly shortened the period in which the filter paper was decomposed. In several cases after four transfers the period was shortened from eight weeks

originally required for cellulose decomposition to three days. The transfer of very small pieces of filter paper or of very small quantities of the liquid portion of the medium failed in each instance to produce growth.

If small pieces of filter paper were smeared on nutrient agar and salt free nutrient agar abundant growth occurred during twenty four hours of incubation at 37°C. If this growth was heavily transfered back to Omeliansky's medium cellulose decomposition again became very slow and required almost as long for decomposition as originglly. This was true even though the cultures were transfered after twelve hours. If these cultures were transfered after 30 to 36 hours cellulose decomposition failed to take place. Transfers made to nutrient broth containing filter paper and incubated at room temperature failed to produce cellulose decomposition.

VIII. pH OF THE FECES OF NORMAL ADULT BIRDS.

PROCEDURE. Samples of feces were collected from the cloaca of normal adult birds in sterile test tubes. The sample was thoroughly emulsified in neutral sterile distilled water. The pH of the sample was then determined by Youden's hydrogen-ion concentration apparatus.

RESULTS.

1.	7.115	28.	8.040	55.	6.690
2.	7.285	29.	6.945	56.	7.615
3.	8.210	30.	6.860	57.	7.370
4.	6.775	31.	6.350	58.	7.370
5.	7.450	32.	6.945	59.	6.435
6.	7.785	33.	6.265	60.	5.585
7.	6.095	34.	6.860	61.	6.180
8.	6.435	35.	7.200	62.	6.350
9.	6.265	36.	7.200	63.	6.945
10.	6.435	37.	6.690	64.	7.115
11.	7.700	38.	6.265	65.	7.115
12.	6.945	39.	6.350	66.	5.840
13.	7.615	40.	7.530	67.	6.690
14.	7.030	41.	6.945	68.	6.860
15.	7.530	42.	6.520	69.	5.925
16.	6.265	43.	6.520	70.	6.860
17.	6.860	44.	6.435	71.	7.285
18.	6.690	45.	6.605	72.	6.945
19.	6.180	46.	6.180	73.	6.350
20.	7.530	47.	5.500	74.	5.585
21.	7.115	48.	5.250	75.	7.200
22.	6.605	49.	6.435	76.	6.945
23.	8.210	50.	7.235	77.	5.500
24.	8.210	51.	6.945	78.	5.500
25.	6.520	52.	7.200	79.	6.095
26.	6.860	53.	6.690	80.	6.520
27.	6.690	54.	6.945	81.	6.435

The pH of the above samples of feces varied from 5.5 to 8.2, the average of the eighty one samples being 6.72 .

IX. DISCUSSION.

Fundamentally there seems to be very little difference in the bacterial flora of the feces of the ten two weeks old normal chicks and the twenty normal adult birds studied. However, there seems to be more stability in the occurrence of Esch. coli and Esch. communior in the feces of the adult birds than in the feces of the chicks. One or the other of these two organisms was absent in the feces of three chicks. Thirty four organisms were found in the feces of the ten chicks while the same number was found in the feces of the twenty hens studied. In health it is probable that there is a tendency in early life to acquire a flora in which Esch. coli and Esch. communior are to predominate.

It seems very apparent that Esch. coli and Esch. communior are the predominating organisms in the bacterial flora of the feces of the normal adult bird as well as being constant inhabitants. The remaining organisms apparently are of little significance and their presence is no doubt governed by certain symbiotic relations that exist from time to time or by chance. Even birds under similar conditions of environment have a wide range of variance in the species of organisms present, excepting of course the colon organisms.

Sal. pullorum was found in the intestinal contents of all of the chicks affected with pullorum disease.

The fact that this organism was found in the intestinal contents of two chicks even though it was not isolated from the organs of these birds would lead one to believe, as suggested by Mallmann (17), that the intestinal contents as well as organs should be cultured when autopsies are held on suspicious chicks. Sal. pullorum was found to be present in the intestinal contents of one diseased adult bird to the extent of 26.4 per cent. Thus it would appear that adult birds may in some instances spread this organism in their droppings. It is not known whether this bird was a reactor to the agglutination test for pullorum disease.

Organisms found in the intestinal contents of the twenty diseased birds consisted chiefly of organisms of the paratyphoid and typhoid group. It seems very likely that the presence of worms or coccidia or both in the intestinal tract may produce a condition in which the percentage occurrence of Esch. coli and Esch. communior is greatly reduced thus allowing the establishment and multiplication of pathogenic organisms. The marked presence of paratyphoid and typhoid organisms in the intestinal contents of the diseased adult birds would lead one to the conclusion that the colon type of organism does much to prevent invasion of the intestinal tract by pathogenic bacteria.

It would appear that possibly some of the acute deaths occurring in coccidiosis and worm infestation may be due to invasion by pathogenic organisms through the

inflamed or broken mucosa and in a few instances may even be due to invasion by the colon organisms themselves.

It is very unlikely that cellulose is decomposed in the intestinal tract of the normal fowl. From the experimental data it would appear that cellulose is not decomposed with any degree of rapidity unless great numbers of cellulose decomposing organisms are present. Since the time required for food to pass through the intestinal tract of the bird is comparatively short it would appear that this time is much too short for cellulose decomposition to take place. It appears that the colon organisms are constant inhabitants of the intestinal tract of normal adult birds and since the intestinal contents are a more favorable medium for the growth of these organisms it would seem that they would tend to inhibit the growth of cellulose decomposing organisms. While it is true that the feces of some birds are semi-fluid in consistency and contain practically no cellulose no accurate estimate can be made of the amount of cellulose individual birds actually ingest.

It is no doubt true that many organisms that invade the intestinal tract of birds cannot be isolated by the procedure used in this study. These organisms are probably of little concern,however, as far as disease in the fowl is concerned.

The average pH of eighty one samples of feces taken from the same number of normal adult birds was 6.72 .

X. CONCLUSIONS.

1. Organisms of the colon group appear to be constant inhabitants of the intestinal tract of mature birds. They represented an average of 60 per cent of the total organisms present in the feces of the twenty adult birds studied.

2. The bacterial flora of the feces of ten two week old chicks studied did not differ fundamentally from that of the adult birds except that there was more or less instability as far as the presence of organisms of the colon group was concerned.

3. In this study all of the ten chicks affected with pullorum disease harbored Sal. pullorum in their intestinal contents.

4. Twenty adult birds affected with enteritis showed a predominence of organisms of the paratyphoid and typhoid group in the intestinal contents and a very marked decrease in the number of colon organisms present.

5. The finding of Sal. pullorum in the intestinal contents of one hen would lead one to believe that some adult birds may carry and spread this organism in their droppings.

6. A large number of samples of feces of adult birds decomposed cellulose when placed in Omeliansky's medium but it is doubtful if cellulose decomposition occurs in the intestinal tract of birds.

7. The average pH of the feces of 81 healthy adult birds was 6.72 .

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